Precipitation of Caprine and Bovine Caseins from Acidic Solutions by Sodium Polyphosphate: Influence of pH and Urea. Utilization for Separation of α_s- and κ-Caseins

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Abstract

The caseins, both caprine (goat) and bovine, are precipitated by relatively low concentrations (approximately 4 mg per 10 ml for a 0.5% casein solution at pH 2.0) of sodium polyphosphate. The casein precipitation is pH dependent (1.5 mg per 10 ml precipitates 50% at pH 2.0, whereas only 0.5 mg is required at pH 3.5), but this pH dependence is much less than that shown by sulfate precipitation of the caseins. κ-Casein in the presence of 2.0 M urea is not precipitated by polyphosphate; this concentration of urea has a neglible effect on the precipitation of α_s-casein. This behavior provides a means for separating α_s- and κ-caseins. Separation is facilitated by reducing the κ-casein to the monomeric form with mercaptoethanol. The caprine and bovine κ-caseins cannot be distinguished in the stabilization of α_s-casein against precipitation with calcium ions.

A previous report (9) has described the precipitation of caseins in acidic solutions by anions, particularly the sulfate ion. The precipitation of the caseins by the divalent sulfate ion appears to be characteristic for divalent anions. These studies have been extended to the precipitation of the caseins by the polyanion polyphosphate,[1] a more general protein precipitant. The influences of pH, urea, and other factors on the precipitation have been studied. Precipitation by polyphosphate in the presence of urea has provided a means for separating α_s- and κ-caseins. This separation has been reported briefly (10). These studies have been done with both bovine and caprine (goat) caseins. The caprine and bovine κ-caseins cannot be distinguished in the stabilization test with the α_s-caseins of either species.

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[1] The term polyphosphate is preferred (6a) for the condensed phosphates available commercially and obtained by heating salts of orthophophate (1), since they are predominantly long-chain or linear olyphosphates. The term metaphosphate is reserved for the condensed cyclic phosphates (6a).

Materials and Methods

Bovine caseins. The preparation of whole casein and α_s- and κ-caseins has been described previously (9).

Caprine caseins. The preparation of whole casein has been reported, as well as the preparation of a crude κ-casein (11). The preparation of relatively pure α_s- and κ-caseins by the use of polyphosphate in urea at pH 3.0 has been reported briefly (10).

Polyacrylamide gel electrophoresis was performed in a vertical cell at pH 9.0 in 4.5 M urea with an acrylamide concentration of 7.0%. In some experiments the caseins were treated with mercaptoethanol (7) before electrophoresis.

Preparation of casein solutions. The preparation of acidic (HCl) solutions of the caseins has been described (9). Essentially, neutral solutions of the caseins are prepared and then rapidly acidified.

Sodium polyphosphate solution. A stock solution of a commercial (Fisher[2]) sodium hexametaphosphate [average number of 7 P atoms per

[2] It is not implied that the USDA recommends the above company or its product to the exclusion of others in the same business.

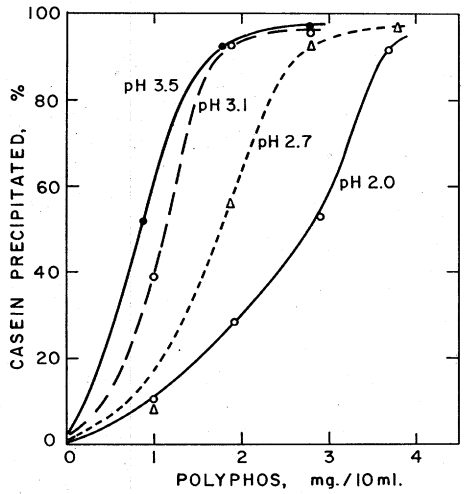

FIG. 1. Influence of pH on the precipitation of 0.4% whole caprine casein by sodium polyphosphate.

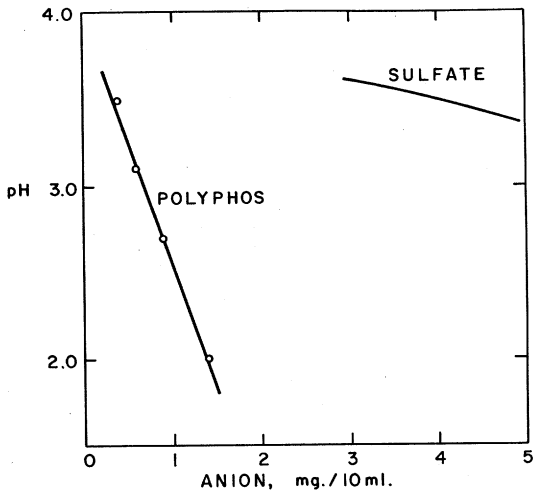

FIG. 2. Relation between pH and the concentration of polyphosphate giving 50% precipitation of caprine whole casein. The data for sulfate are taken from Reference 9. Weights of the anions are those of the sodium salts.

chain (2)[1]] was prepared at a concentration of 1.0%. It was acidified to a pH of 3.0 with a small amount (about 0.3 ml per g) of 3 N HCl. The concentration used in the experiments is expressed in mg per 10 ml.

Urea solution. A 6.6 M solution was acidified to pH 3.0 with 3 N HCl (about 1 ml per 100 ml). A fresh acidified solution was prepared weekly, since the solution became less acid quite rapidly.

Results

The influence of pH on the course of precipitation of caprine whole casein by polyphosphate is shown in Fig. 1. A similar influence of pH was observed with bovine whole casein. The curves obtained are almost identical. The relative insensitivity of the polyphosphate concentration giving 50% precipitation in relation to pH, compared with precipitation with a divalent ion-like sulfate (9), is shown in Fig. 2.

The influence of urea on the precipitation of caprine α_s- and κ-caseins at pH 3.0 by polyphosphate is shown in Fig. 3. The curve for whole casein is intermediate between the curves for α_s- and κ-caseins. The curve for β-casein is intermediate between the curves for α_s-casein and whole casein. Very similar results were obtained for the bovine caseins.

In view of the marked dissimilarity of the influence of urea on the solubility of α_s- and κ-casein, a possible method for separating these two caseins appeared likely. The goat κ-casein prepared by the sulfuric acid–urea method contained considerable α_s-casein (11). The observations on the effect of urea were utilized in the following method for separating κ- and α_s-caseins.

Five grams of caprine (or bovine) κ-casein prepared by the sulfuric acid–urea method was dissolved in 100 ml water at pH 7.5 and 1 ml mercaptoethanol was added. After 15 min 100 ml of 6.6 M urea was added, the solution acidified to pH 3.0 with 3 N HCl and, finally, 12 ml of 1% sodium polyphosphate was added. The flocculent precipitate which forms is α_s-casein. The protein remaining in solution is κ-casein. This was precipitated with 75 g ammonium sulfate. The precipitates were suspended in water, dissolved, and neutralized with NaOH, dialyzed, and freeze-dried. The electrophoretic patterns obtained in polyacrylamide gel at pH 9.0 for both preparations are shown in Fig. 4. The mercaptoethanol, which reduces the κ-casein to the monomer, is necessary for the cleanest separation. Without it the polyphosphate precipitate is colloidal and difficult to sediment in the centrifuge. The caprine κ-casein will stabilize the caprine α_s-casein in the presence of calcium ions (11). It will also stabilize bovine α_s-casein as effectively as does bovine κ-casein (10). The comparative results with the two κ-caseins by the standard stabilization test (8) are shown in Fig. 5.

Discussion

Polyphosphate is an effective precipitant of the caseins, as it is of other proteins (11). Precipitation by polyphosphate is influenced by pH to some extent (Fig. 1 and 2), but not nearly to the extent that precipitation by sul-

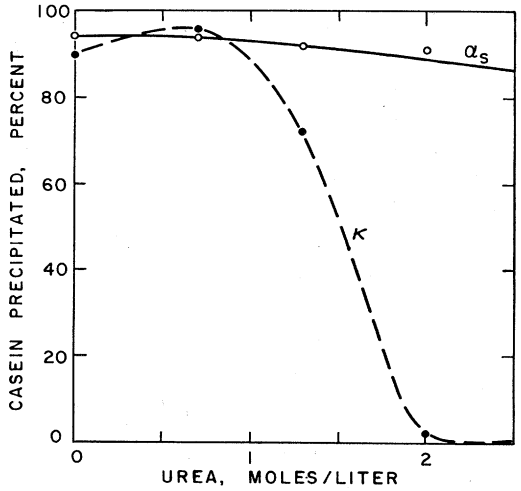

FIG. 3. Influence of urea on the precipitation of caprine caseins (0.4%) by polyphosphate (3.0 mg per 10.0 ml) at pH 3.0. ○ = α_s-casein; ● = κ-casein.

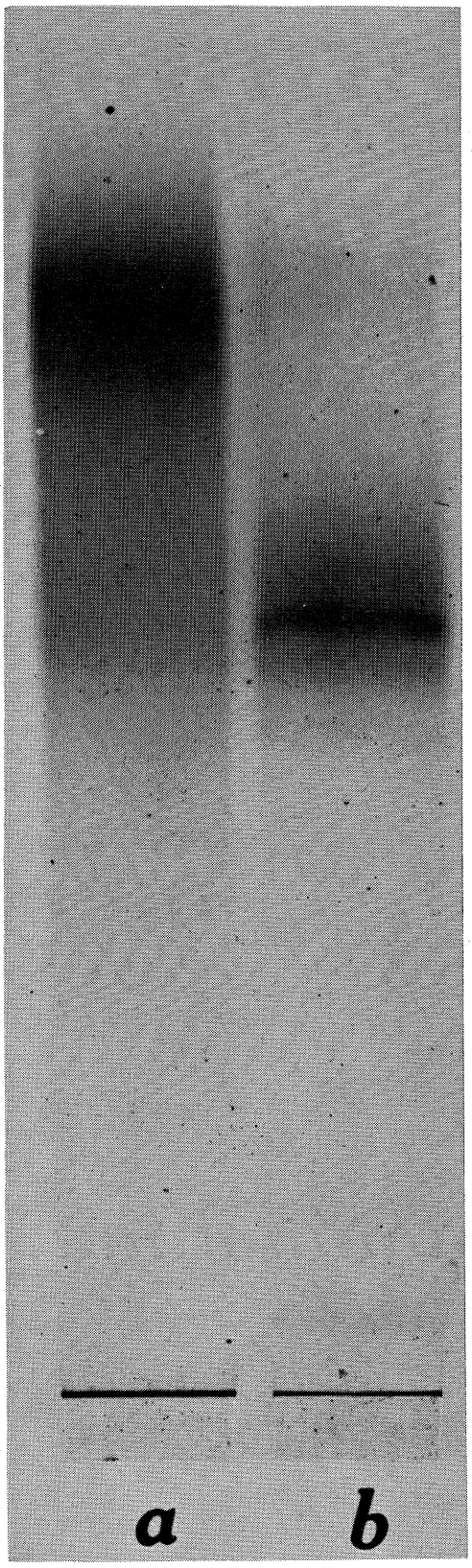

FIG. 4. Electrophoresis in polyacrylamide gel at pH 9.0, with mercaptoethanol present, of goat α_s-casein (a) and κ-casein (b), separated by the polyphosphate procedure.

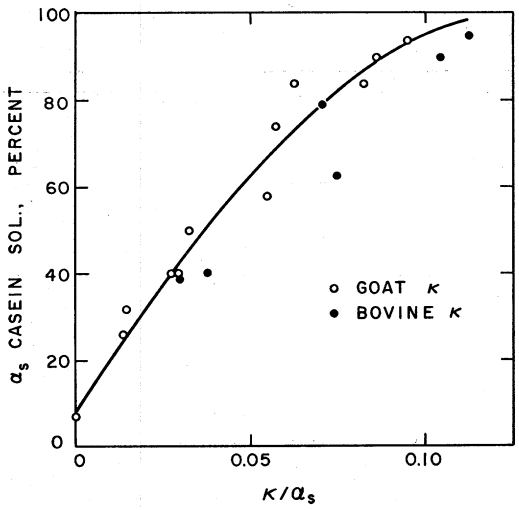

FIG. 5. Stabilization of bovine α_s-casein with caprine and with bovine κ-casein.

fate is influenced (Fig. 2). This probably reflects the stronger binding of polyphosphate to proteins, leading to neutralization of the charge on the protein (9) and the cross bonding between protein molecules that the extended polyphosphate molecules bring about. The binding of polyphosphate to proteins has been found to correlate with the number of positively charged groups on the proteins (4). Doubling the amount of polyphosphate required for 50% precipitation of casein between pH 3.5 and 2.3 parallels the amount of HCl required to reach the respective pH values (1).

The striking difference in the influence of urea on polyphosphate precipitation of κ-casein and α_s-casein appears to reside in a specific interaction between the κ-casein and the polyphosphate. This conclusion is supported by the influence of urea on the isoelectric precipitation (pH 4.7) of κ- and α_s-caseins. In this instance the precipitation of both caseins is influenced equally by urea, to about the same degree as α_s-casein in the polyphosphate experiments. This effect of polyphosphate is not unique for this compound, but apparently is an effect of polyanions, since the polysulfate, heparin, shows a similar effect on κ- and α_s-caseins in urea.

The polyphosphate method has been equally effective in separating mixtures of κ- and α_s-caseins, whether bovine or caprine.

The preparations obtained by this procedure are quite pure, but the use of a technique like

chromatography would probably be desirable for complete purification. Since the minor band just ahead of the major κ-casein band in the goat preparation (Fig. 4) is also transformed by rennin (11), this must be considered a κ-casein component. Chromatography on DEAE-cellulose at pH 7.0 with a NaCl gradient has given some separation of these major and minor κ-caseins (6). These components account for the major band and the minor band observed in para-κ-casein (11).

The investigation of the influence of urea on polyphosphate precipitation of caseins (Fig. 3) was extended to include whole and β-casein. Whole casein in its precipitation was intermediate between κ- and α_s-caseins, and β-casein was intermediate between whole casein and α_s-casein. The position of whole casein presumably reflected its content of κ-casein and suggested that polyphosphate precipitation in urea might be a means of separating κ-casein from whole casein. The procedure applied to whole goat casein was not effective; the soluble portion invariably contained some β-casein, together with the κ-casein. This reaction, however, under somewhat different conditions (0.1 M acetic acid, 3.0 M urea, 0.1% polyphosphate), has been the means of separating bovine α_s-casein from κ- and β-caseins (3).

Some preparations of caprine α_s-casein, although apparently free of κ-casein, based on gel electrophoresis, precipitated not at all or incompletely with calcium ions (0.01 M). Treatment with rennin, however, led to increased precipitation with calcium ions and usually the appearance of a faint para-κ-casein band. Thus, what appeared to be anomalous α_s-casein (10) is probably due to contamination with small amounts of κ-casein.

The effectiveness of mercaptoethanol treatment of κ-casein in giving improved precipitation of the contaminating α_s-casein is probably due to transformation of the κ-casein to the monomeric form. This form presumably has less tendency to associate physically, in a nonspecific way, with the α_s-casein (the specific association between α_s- and κ-caseins leading to stabilization of the α_s-casein in the presence of calcium ions apparently is equally effective whether the κ-casein is in polymeric or monomeric form, since both forms are equally good stabilizers). The advantage of having κ-casein in its monomeric form to reduce association with the other caseins has been observed also in chromatography (5).

The ability of caprine κ-casein to stabilize bovine α_s-casein must indicate a close relationship to the bovine κ-casein. The degree of this relationship will need to be established by amino acid analysis. Some difference is anticipated, since the caprine κ-casein (reduced monomeric form) has a somewhat greater electrophoretic mobility than bovine κ-casein. Also, polymerization of the caprine κ-casein appears to lead to polymers of discrete sizes (11), whereas the bovine κ-casein polymer is very heterogeneous. An important difference in the two κ-caseins is the number of minor κ components; caprine κ-casein apparently has only one, whereas bovine κ-casein has about seven. Furthermore, the caprine κ-casein has much less sialic acid (0.3% versus 2.0%).

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